Summary of Safety and Effectiveness Data

1. General Information

1.1. Device Generic Name: In vitro nucleic-acid amplification test for qualitatively detecting *Hepatitis C virus* (HCV) RNA in human serum or plasma from blood collected in EDTA

1.2 Device Trade Name: AMPLICOR® HCV Test, v2.0

1.3 Applicant's Name and Address:

Roche Molecular Systems, Inc. (RMS)
4300 Hacienda Drive
Pleasanton, CA 94588

1.4 Premarket Approval Application (PMA) Number: P000010

1.5 Date of Panel Recommendation: July 28, 2000

1.6 Date of Notice of Approval : July 5, 2001

2. Indications for Use

The AMPLICOR™ HCV Test, v2.0 is an *in vitro* diagnostic, nucleic-acid amplification test for qualitative detection of HCV RNA in human serum or plasma from blood collected in EDTA. This test detects by reverse-transcribing target HCV RNA into complementary DNA (cDNA), amplifying cDNA by polymerase chain reaction (PCR), hybridizing amplified cDNA with an oligonucleotide probe that binds enzyme, and catalyzing conversion of substrate to a colored product that is recognized by a microwell plate reader. The AMPLICOR HCV Test, v2.0 is indicated for patients who have evidence of liver disease and antibody evidence of HCV infection, and who are suspected to be actively infected with HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active HCV infection.

3. Contraindications

None known.

4. Warnings and Precautions

The AMPLICOR HCV Test, v2.0 is for *in vitro* diagnostic use only. Refer to labeling for a listing of other warnings and precautions.

5. Device Description

5.1. Test Principles

The AMPLICOR HCV Test, v2.0 is based on five major processes:

- specimen preparation
- reverse transcription to generate cDNA from target HCV RNA and HCV Internal Control (HCV IC) RNA
- PCR amplification of target cDNAs by using HCV-specific primers
- hybridization of amplified cDNAs to target-specific oligonucleotide probes
- colorimetric detection of the probe-bound amplified cDNAs.

The AMPLICOR HCV Test, v2.0 permits simultaneous reverse transcription and PCR amplification of HCV and HCV IC target RNAs. The Master Mix reagent contains a primer pair that is specific for both HCV and HCV IC RNAs. Detection of amplified DNA is performed using target-specific oligonucleotide probes that permit independent identification of HCV amplicon and HCV IC amplicon.

5.1.1. Specimen Preparation

HCV RNA is isolated directly from serum or EDTA plasma by lysing virus particles with a chaotropic agent. HCV IC RNA, introduced into each specimen with Lysis Reagent, serves as an extraction and amplification control for each processed specimen. HCV and HCV IC RNAs are precipitated by using alcohol and then resuspended in Specimen Diluent.

5.1.2. Enzyme and Target Selection

Enzyme: Reactions are catalyzed by thermostable, recombinant *Thermus thermophilus* DNA Polymerase (r*Tth* pol). In the presence of manganese ion (Mn^{2+}) and the appropriate buffer, r*Tth* pol has reverse transcriptase and DNA polymerase activities. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture.

Target selection: Selection of primers and probe was critical for AMPLICOR HCV Test, v2.0 detection of all recognized genotypes (see Non-Clinical Performance section of the Package Insert). Accordingly, selection of the target RNA sequence was based on identifying an HCV-genome region that was maximally conserved among genotypes. HCV RNA sequences are most conserved in the 5' untranslated region (UTR) . The AMPLICOR HCV Test, v2.0 uses primers KY78 and KY80 to amplify a 5' UTR sequence of 244 nucleotides. HCV RNA sequence corresponding to these primers and the capture probe are located in the most conserved 5' UTR domains.

5.1.3. Reverse Transcription

Processed specimens are added to the amplification mixture in reaction tubes, in which reverse transcription and PCR amplification occurs. The downstream or antisense primer (KY78) is biotinylated at its 5' end; the upstream or sense primer (KY80) is not biotinylated. The reaction mixture is heated in the thermal cycler to allow specific annealing of the downstream primer to target HCV and HCV IC RNAs. In the presence of Mn^{2+} and excess deoxynucleoside triphosphates (dNTPs), including

deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of thymidine) triphosphates, r*Tth* pol extends the annealed primer to form cDNA.

5.1.4. Target Amplification

Following reverse transcription of target HCV and HCV IC RNAs, the reaction mixture is heated to denature RNA:cDNA hybrids and expose sequences that anneal with the primers. As the mixture cools, the upstream primer (KY80) anneals specifically to the cDNA strand representing each target RNA, r*Tth* pol extends the primer, and a second DNA strand is synthesized. This completes the first cycle of PCR, yielding a double-stranded DNA copy of each RNA target region (HCV and HCV IC).

The reaction mixture is heated again to separate the double-stranded DNA and expose the primer-annealing sequences. As the mixture cools, primers KY78 and KY80 anneal to target DNA. The r*Tth* pol enzyme, in the presence of Mn^{2+} and excess dNTPs, extends the annealed primers along the target templates to produce a 244 base pair double-stranded DNA "amplicon." The thermal cycler automatically repeats this process for 37 cycles, with each cycle intended to double the amount of amplicon DNA. Amplification occurs only in the region of the HCV genome between the primers; the entire genome is not amplified.

5.1.4.1. Amplification of HCV Internal Control (HCV IC) cDNA

In enzyme-based amplification processes such as PCR, inhibitors that may be present in the clinical specimen can reduce amplification efficiency. The HCV IC has been added to the AMPLICOR HCV Test, v2.0 to permit identification of processed specimens containing substances that may interfere with PCR amplification or specimens in which HCV RNA may have been lost during sample processing. The HCV IC is an RNA transcript with primer-annealing regions identical to those in the HCV genome, a randomized internal sequence of similar length and base composition as the HCV target sequence, and a unique probe-binding region that differentiates HCV IC amplicon from HCV amplicon. These features were selected to ensure equivalent amplification of HCV IC and HCV target RNAs. The HCV IC is introduced into each specimen with the Lysis Reagent and serves as an extraction and amplification control for each processed specimen.

5.1.4.2. Selective Amplification

Selective amplification of target nucleic acid from the clinical specimen is achieved in the AMPLICOR HCV Test, v2.0 by the use of AmpErase® (uracil-N-glycosylase) and deoxyuridine triphosphate (dUTP). AmpErase recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing thymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon from this assay due to the use of deoxyuridine triphosphate in place of thymidine triphosphate

as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine.

Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase prior to amplification of the target DNA. AmpErase, which is included in the Master Mix reagent, catalyzes cleavage of deoxyuridine-containing DNA at deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. AmpErase is inactive at temperatures above 55°C (i.e., throughout the thermal cycling steps) and therefore does not destroy target amplicon. Following amplification, any residual AmpErase is denatured by the addition of Denaturation Solution, thereby preventing the degradation of any target amplicon. AmpErase in the AMPLICOR HCV Test, v2.0 has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing HCV amplicon per PCR.

5.1.5. Hybridization Reaction

Following PCR amplification, and after the addition of Denaturation Solution to the reaction tubes, the HCV amplicon and the HCV IC amplicon are chemically denatured to form single-stranded DNA. Aliquots of denatured amplicon are then transferred to separate wells of microwell plates (MWP) coated with either an HCV-specific (KY150) or HCV IC-specific (SK535) oligonucleotide probe. The biotin-labeled HCV and HCV IC amplicon are hybridized to the target-specific oligonucleotide probes bound to the wells of the MWP. This hybridization of amplicon to the target-specific probe increases the overall specificity of the test.

5.1.6. Detection Reaction

Following the hybridization reaction, the MWP is washed to remove unbound material and Avidin-Horseradish Peroxidase Conjugate is added to each well of the MWP. The Avidin-Horseradish Peroxidase Conjugate binds to the biotin-labeled amplicon which is hybridized to the target (HCV or HCV IC) specific oligonucleotide probes bound to the MWP. The MWP is washed again to remove unbound conjugate and a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) is added to each well. In the presence of hydrogen peroxide, the bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex. The reaction is stopped by addition of a weak acid and the absorbance is measured at a wavelength of 450 nm (A_{450}) using a microwell plate reader.

5.2. Kit Configuration and Components

The AMPLICOR HCV Test, v2.0 consists of five separately packaged kits containing the controls and reagents required for specimen preparation, reverse transcription, and PCR amplification and detection of HCV and Internal Control target RNA (Table 1).

Table 1: AMPLICOR Ô HCV Test, v2.0 Kits

AMPLICOR HCV Specimen Preparation Kit, version 2.0	96 Tests	P/N: 21111086; US: 83126
AMPLICOR HCV Controls Kit, version 2.0	8 Sets	P/N: 21111175; US: 83131
AMPLICOR HCV Amplification Kit, version 2.0	96 Tests	P/N: 21111094; US: 83127
AMPLICOR HCV Detection Kit, version 2.0	96 Tests	P/N: 21118439; US: 833373
AMPLICOR Internal Control Detection Kit	96Tests	P/N: 20751952; US: 83068

The components of each kit are listed below (Table 2).

Table 2: Components of the AMPLICOR â HCV Test, v2.0

KIT COMPONENT	*FORMULATION*
AMPLICOR HCV Specimen Preparation Kit, version 2.0	
(1) HCV Lysis Reagent, v2.0 **HCV LYS, v2.0**	Tris-HCl buffer containing 68% guanidine thiocyanate, 3% dithiothreitol and <1% glycogen, 8 x 6.9 mL
(2) HCV Specimen Diluent, v2.0 **HCV DIL, v2.0**	Tris-HCl buffer containing, <0.005% poly rA RNA (synthetic), EDTA and 0.05% sodium azide, 8 x 4.8 mL
(3) HCV Internal Control, v2.0 **HCV IC, v2.0**	A solution containing <0.001% non-infectious *in vitro* transcribed RNA (microbial) encoding HCV primer binding sequences and a unique probe binding region, <0.005% poly rA RNA (synthetic), EDTA, and 0.05% sodium azide, 8 x 0.1 mL
AMPLICOR HCV Controls Kit, version 2.0	
(4) Negative Plasma (Human) **NHP**	Human plasma non-reactive by FDA licensed tests for antibody to HCV, antibody to HIV-1 and HIV-2, HIV p24 antigen and HBsAg, and containing 0.1% ProClin® 300, 8 x 0.6 mL
(5) HCV (-) Control, v2.0 **HCV (-) C, v2.0**	A solution containing <0.005% poly rA RNA (synthetic), EDTA and 0.05% sodium azide, 8 x 0.1 mL
(6) HCV (+) Control, v2.0 **HCV (+) C, v2.0**	A solution containing <0.001% non-infectious *in vitro* transcribed RNA (microbial) encoding HCV sequences, <0.005% poly rA RNA (synthetic), EDTA and 0.05% sodium azide, 8 x 0.1 mL

Table 2: (continued) Components of the AMPLICOR â HCV Test, v2.0

AMPLICOR HCV Amplification Kit, version 2.0	
(7) HCV Master Mix, v2.0 **HCV MMX, v2.0**	A bicine buffered solution containing 16% DMSO, glycerol, < 0.01% r*Tth* DNA Polymerase (r*Tth* pol, microbial), potassium acetate, < 0.001% dATP, dCTP, dGTP, and dUTP, <0.005% KY78 and KY80 primers (one is biotinylated), < 0.01% AmpErase® (microbial) and 0.05% sodium azide, 8 x 0.7 mL
(8) HCV Manganese Solution, v2.0 **HCV Mn^{2+}, v2.0**	A solution containing <2% manganese, acetic acid, Amaranth dye, 0.05% sodium azide , 8 x 0.1 mL
KIT COMPONENT	***FORMULATION***
AMPLICOR HCV Detection Kit, version 2.0	
(9) HCV Microwell plate, v2.0 **HCV MWP, v2.0**	MWP coated with HCV-specific DNA Probe (KY150). Twelve, 8-well strips in one resealable pouch with desiccant
(10) Denaturation Solution **[1] DN**	A solution containing 1.6% sodium hydroxide, EDTA and thymol blue , 1 x 12 ml
(11) HCV Hybridization Buffer **[2] HCV HYB**	A sodium phosphate solution containing < 0.2% solubilizer and < 25% sodium thiocyanate, 1 x 20 mL
(12) Avidin-Horseradish Peroxidase Conjugate **[3] AV-HRP**	A Tris-HCl buffered solution containing <0.001% avidin-horseradish peroxidase conjugate, bovine serum albumin (mammalian), Emulsit 25, 0.1% phenol, 1% ProClin® 150, 1 x 12 mL
(13) Substrate A **[4A] SUB A**	A citrate solution containing 0.01% hydrogen peroxide, 0.1% ProClin 150, 1 x 12 mL
(14) Substrate B **[4B] SUB B**	0.1% 3,3',5,5'-Tetramethylbenzidine (TMB) in 40% dimethylformamide (DMF), 1 x 3 mL
(15) Stop Reagent **[5] STOP**	Contains 4.9% Sulfuric acid, 1 x 12 mL

(16) 10X Wash Concentrate **10X WB**	A solution containing < 2% phosphate buffer, < 9% sodium chloride, EDTA, < 2% detergent, 0.5% ProClin 300, 2 x 90 mL

Table 2 (continued). Components of the AMPLICOR Ô HCV Test, v2.0

KIT COMPONENT	*FORMULATION*
AMPLICOR Internal Control Detection Kit	
(17) Internal Control Microwell Plate **IC MWP**	MWP coated with IC-specific DNA probe (SK 535), Twelve, 8-well strips in one resealable pouch with desiccant, 1 x 96 tests
(18) Avidin-Horseradish Peroxidase Conjugate **[3] AV-HRP**	A Tris-HCl buffered solution containing <0.001% avidin-horseradish peroxidase conjugate, bovine serum albumin (mammalian), Emulsit 25, 0.1% phenol, 1% ProClin® 150, 1 x 12 mL
(19) Substrate A **[4A] SUB A**	A citrate solution containing 0.01% hydrogen peroxide and 0.1% ProClin® 150, 1 x 12 mL
(20) Substrate B **[4B] SUB**	Contains 0.1% 3,3′,5,5′-tetramethylbenzidine (TMB) in 40% dimethylformamide (DMF), 1 x 3 mL
(21) Stop Reagent **[5] Stop**	Contains 4.9% Sulfuric acid, 1 x 12 mL
(22) 10X-Wash Concentrate **10 X WB**	A solution containing <2% phosphate buffer, <9% sodium chloride, EDTA, <2% detergent and 0.5% ProClin 300, 2 x 90 mL

6. Alternative Practices and Procedures

6.1. Biochemical Methods

Alanine aminotransferase (ALT) is a marker of hepatocellular damage and its concentration in serum is often elevated during viral hepatitis. Prior to identification of HCV and subsequent development of tests for detecting anti-HCV, ALT was used as a surrogate marker for "parenterally transmitted non-A, non-B" hepatitis. As such, ALT was a non-specific test for HCV infection. More recently, and in the absence of a more specific or virologic test, changes in ALT concentration were used to assess effectiveness of antiviral therapy for chronic hepatitis C. While it is still used in assessing severity of chronic hepatitis C without therapy, ALT concentration poorly predicts the presence or absence of histopathologic changes in the liver.

6.2. Serologic or Virologic Methods

Currently, there are only a few methods for detecting evidence of HCV infection. Such evidence can be indirect (e.g., detection of an immunologic response) or direct (e.g., detection of the virus or its macromolecules).

6.2.1. Testing for Antibodies to HCV (anti-HCV)

Anti-HCV assays yield indirect evidence of HCV infection but cannot determine if the infection is active (i.e., HCV is actively replicating) or inactive. Furthermore, current antibody tests do not yield temporal information: results from a single specimen cannot be used to determine if an infection is acute or chronic.

Enzyme immunoassay (EIA) is the most widely used method for detecting evidence of HCV infection and is usually a first step in laboratory diagnosis of hepatitis C. Current EIAs (FDA-licensed as version 2 or 3) detect antibodies to antigens representing core (nucleocapsid) and certain non-structural HCV proteins. EIAs are readily performed but less than optimally specific. Therefore, package inserts for currently licensed EIAs advise supplemental testing to ensure that a "repeatedly reactive" result accurately indicates detection of antibodies to HCV.

Strip immunoassay (SIA) is the most widely used method for supplemental detection of antibody evidence of HCV infection and, currently, version 2 is licensed. CDC and its consultants[2] have also recommended using appropriate HCV RNA assays for supplemental evidence of infection (see below; Virologic Methods).

6.2.2. Virologic Methods

Virus isolation (propagation) cannot currently be used for clinical specimens: HCV is not known to replicate in cell culture. It is also not practical to inoculate susceptible animals (the only well-characterized model of HCV infection uses chimpanzees, *Pan troglodytes*).

Nucleic-acid detection: during recent years, analytically sensitive methods for detecting DNA or RNA were developed that have enabled practical detection or quantitation of the RNA genome inside HCV particles that circulate in the blood. These methods have been widely used for research applications, including detection of HCV RNA. Many are commercially available, often as generic "kits" that a laboratory can adapt for detection of particular analyte like HCV RNA.

All such methods are based on hybridization between reagent nucleic acid molecules and DNA or RNA "target" in the sample and subsequent production of an identifiable "signal." These methods are frequently categorized as "nucleic-acid amplification tests" (NAT), terminology that is somewhat misleading because of wide variation in technological approaches and in analytical sensitivity. Typically, "target amplification" (polymerase-catalyzed production of nucleic acid representing the target, followed by generation of production-indicating signal) is analytically more sensitive than "signal

amplification" (enhanced production of signal that indicates direct hybridization with target). Examples of target amplification methods include PCR, nucleic acid sequence based amplification (NASBA), transcription mediated amplification (TMA), self-sustained sequence replication (3SR, a non-commercial predecessor to NASBA and TMA), strand displacement amplification (SDA), ligase chain reaction (LCR), and Qβ replicase (QβR). Examples of target amplification methods include branched chain DNA (bDNA) and hybrid capture (HC).

Assays for HCV RNA in the blood are intended to provide information about the viremic status of HCV-infected individuals, as demonstrated in peer-reviewed scientific literature. Other literature suggests that HCV viremia is a reliable marker of active HCV replication in the liver. Therefore, HCV RNA assays have been used in research for direct evidence of active HCV infection, in diagnostic or monitoring modes. The monitoring mode includes assessment of antiviral-therapy effects.

Several HCV RNA assays are commercially available but, other than the COBAS AMPLICOR™ HCV Test, v2.0 and AMPLICOR® HCV Test, v2.0, none have been FDA-approved for in vitro diagnostic use. Furthermore, none are FDA-approved for monitoring HCV infections (including monitoring of response to therapy for hepatitis C) or FDA-licensed for use in determining safety of blood, blood products, or tissue. Like the COBAS AMPLICOR HCV Test, v2.0 and the AMPLICOR® HCV Test, v2.0, other assays for HCV RNA often use reagent nucleic acids representing 5' UTR sequences that are highly conserved among HCV genotypes (to optimize sensitivity) but distinct from other known nucleotide sequences (in an effort to optimize specificity).

7. Marketing History

The AMPLICOR HCV Test, v2.0 has not been withdrawn from marketing in any country for reasons related to safety or effectiveness. It is currently available for commercial marketing in the following countries:

- Argentina
- Austria
- Australia
- Belarus
- Belgium
- Chile
- Cyprus
- Czech Republic
- Denmark
- Ecuador
- Egypt
- Finland
- France
- Germany
- Greece
- Hong Kong
- Hungary
- Iceland
- India
- Indonesia
- Ireland
- Israel
- Japan
- Korea
- Kuwait
- Lebanon
- Luxembourg
- Malaysia
- Malta
- Mexico
- Netherlands
- New Zealand
- Norway
- Oman
- Pakistan
- Paraguay
- Philippines
- Poland
- Qatar
- Russia
- Saudi Arabia
- Slovenia
- South Africa
- Spain
- Sweden
- Switzerland
- Syria
- Turkey
- United Arab Emirates
- United Kingdom
- Venezuela

8. Potential Adverse Effects of the Device on Health

8.1. Potential Adverse Effects for Patients

Possibilities for erroneous test results are inherent in all *in vitro* diagnostic products. An erroneous result can then lead to an adverse effect for the tested patient. Any current test for evidence of HCV infection, including those for detecting antibodies to HCV, can yield an inaccurate result, or a result that is inaccurately reported to the physician or inaccurately interpreted by the physician. The possibility of an erroneous AMPLICOR HCV Test, v2.0 result exists due to poor specimen quality (e.g., sample contamination or inadequate storage), potential malfunction of a test component, an equipment error, or an operator error. Because HCV RNA is the only current practical marker of active infection, an inaccurate result or interpretation can adversely affect a patient unless other information enables the physician to recognize the inaccuracy.

A ***False Positive*** result (i.e., a AMPLICOR HCV Test, v2.0 Positive result when HCV RNA was not present in the specimen) could lead to a misdiagnosis of active HCV infection. This misdiagnosis could lead to unnecessary diagnostic procedures (e.g., liver biopsy, which has associated risks and discomfort) or psychological trauma to the patient (e.g., concern about a chronic infection that can have severe disease consequences). While misdiagnosis could also lead to unnecessary treatment (current pharmacologic regimens for chronic hepatitis C are less than optimally efficacious and are associated with significant side effects), current practice recommendations include additional HCV RNA testing (quantitative and genotyping) for most patients before therapy begins.

A ***False Negative*** result (i.e., a AMPLICOR HCV Test, v2.0 Negative result when HCV RNA was present) could lead to a misdiagnosis of cleared, or inactive, HCV infection. This misdiagnosis could lead to unnecessary procedures in consideration of other diagnoses; a delay in, or failure to perform, appropriate additional diagnostic procedures (e.g., to determine the state of active HCV infection or severity of HCV-associated liver disease); or a delay in, or failure to provide, appropriate treatment.

Data submitted by RMS and in peer-reviewed scientific literature provided reasonable assurance of AMPLICOR HCV Test, v2.0 safety and effectiveness. That is, adverse effects could be severe but the potential for a false result appeared to be very low (and exceeded by the likelihood of benefit from an accurate result).

8.2. Potential Adverse Effects for Laboratory Personnel

There is also potential for adverse effects, from an AMPLICOR HCV Test, v2.0 component, on the health of laboratory personnel performing this assay. One component, Negative Plasma (Human), should be considered potentially infectious because it contains human-source materials. However, before the component is manufactured, these materials must be non-reactive for HBsAg and for antibodies to HCV, HIV-1, and HIV-2.

This potential risk occurs with many other *in vitro* diagnostic products. Appropriate warnings are included in AMPLICOR HCV Test, v2.0 vial and kit labels and in the Package Insert. Most laboratory personnel implement standard good laboratory practices and are protected from hepatitis B due to vaccination or past infection. Therefore, this risk is well known to laboratory personnel and its potential is considered to be minimal.

9. Summary of Non-Clinical Studies

RMS laboratories performed studies to assess analytical sensitivity and specificity of the AMPLICOR HCV Test, v2.0. Those studies that contributed to the determination of safety and effectiveness are summarized in the Non-Clinical Performance section of the AMPLICOR HCV Test, v2.0 Package Insert. Certain findings are also summarized below.

9.1. Limits of Detection (LODs)

LODs were determined by testing multiple specimens containing very low concentrations of the WHO International Standard for HCV genotype 1 RNA*. This WHO standard was diluted in HCV-negative serum and in HCV-negative EDTA plasma to concentrations of 100, 75, 60 and 50 IU/mL. Each concentration was tested with the AMPLICOR HCV Test, v2.0 in replicates ranging from 80 to 120. LODs were determined as the lowest HCV RNA concentration that resulted in ≥ 95% of results yielding $A_{450} \geq 1.0$, the cutoff for Positive results with the AMPLICOR HCV Test, v2.0. By this criterion, the LOD for HCV RNA in serum was 2.0 $\log_{10}$ IU/mL, or 100 IU/mL. The LOD for HCV RNA in EDTA plasma was 1.7 $\log_{10}$ IU/mL, or 50 IU/mL.

* The WHO International Standard for HCV genotype 1 RNA is available, as NIBSC code 96/790, from the National Institute for Biological Standards and Control, London, UK. IU designates International Units of this standard, which contains 10^5 IU/mL of genotype 1 HCV RNA as defined by consensus studies.

Here and in the AMPLICOR HCV Test, v2.0 Package Insert, *IU* designates virion or subgenomic HCV RNA that has been quantified with reference to the WHO Standard. *IU*/mL affords a standardized approach to indicating HCV RNA concentration but it is not known if *IU*/mL accurately reflects the HCV RNA concentration of any particular specimen. Available data indicate that 1 IU corresponds to > 1 HCV RNA molecule and that this number of molecules varies according to quantifying methods (which are imprecise with a single determination) and other variables:

Source of data	Type of RNA	How quantified	Quantifier	Conversion factor
Consensus	HCV (virion)	End-point dilution: qualitative assays	Copies	1 IU ≈ 1.8 copies
		Quantitative assays	Copies or genome equivalents	1 IU ≈ 6.6 copies
Roche Molecular Systems (unpublished)	Subgenomic transcript of cloned HCV cDNA	UV spectroscopy	A_{260}-molecules	1 IU ≈ 2.7 A_{260} molecules (95% CI, 2.6-2.8)

While RMS has demonstrated similar conversion factors for certain RNAs representing HCV genotypes 2-6, it is not known if quantitation with reference to the WHO Standard is affected by genotype, strain characteristics (including RNA structure and quasispecies), or concentration of HCV RNA. For example, IU-to-copy ratios for low concentrations of HCV RNA may be different than those for high concentrations of HCV RNA.

Specimens yielding a result in the Equivocal Zone were not repeat tested (per Interpretation of Results section of the Package Insert), so a final result was not obtained for these specimens. If repeat testing were done, it is reasonable to assume that frequencies of Positive results would increase, possibly resulting in lower LODs. By excluding Equivocal results, for example, the 95% threshold of Positive results was reached at 1.7 $\log_{10}$ IU/mL (50 IU/mL) for serum.

9.2. Detection of HCV Genotypes

Quantified subgenomic RNAs, transcribed from cloned cDNAs, were used to approximate analytical sensitivity of the AMPLICOR HCV Test, v2.0 for five HCV genotypes: 1, 2, 3, 4, and 5. (Genotypes of seven viruses, including two representatives each of genotypes 1 and 2, were determined by using research methods that have not been evaluated or approved by FDA; however, genotyping by many such methods is generally recognized to be accurate whereas many subtyping techniques vary in accuracy.) Each RNA transcript, consisting of the 5'-untranslated and core regions, was quantified by spectrophotometry (A_{260}) and then diluted to three different concentrations in HCV Specimen Diluent, v2.0. Twenty-four replicates of each concentration were tested by the AMPLICOR HCV Test, v2.0. The number of Positive results at each concentration was determined. Positive result frequencies were similar for these subgenomic RNAs representing genotypes 1–5.

Another study evaluated the sensitivity of the AMPLICOR HCV Test, v2.0 under research laboratory conditions to determine if it would yield Positive results with 65 clinical specimens

containing HCV strains that represented the six recognized genotypes. The majority of these specimens were from patients in an antiviral efficacy study and were assumed to contain HCV RNA concentrations that were representative of those patients for whom the AMPLICOR HCV Test, v2.0 is indicated. All 65 specimens yielded Positive results.

9.3. Cross Contamination

Two studies were done under controlled laboratory conditions to assess the potential cross contamination rate of the AMPLICOR HCV Test, v2.0. In 22 runs of the AMPLICOR HCV Test, v2.0, multiple operators tested replicates of plasma specimens containing clinically-pertinent concentrations of HCV RNA (6.5 $\log_{10}$ *IU*/mL) that alternated with replicates of an HCV-negative specimen. Combined results indicated a cross contamination rate of 1.2% (3/241).

The potential for cross contamination was also examined in two reproducibility studies performed under clinical laboratory conditions with lower concentrations of HCV RNA. In the first study, samples with [HCV RNA] ≤ 2.3 $\log_{10}$ *IU*/mL were tested. Among 532 HCV-negative serum specimens, 531 yielded negative results, and 1 (0.2%) yielded an Equivocal result, which may or may not have indicated cross contamination. Among 537 HCV-negative plasma specimens, 3 (0.6%), yielded Equivocal results and 2 (0.4%) yielded Positive results, for a cross contamination rate between 0.4% and 0.9%. In the second study, with 153 specimens at [HCV RNA] ≤ 4.7 $\log_{10}$ *IU*/mL, a cross contamination rate of 0.7% (1/153) was shown .

9.4. Cross-reactivity with or Interference from Other Microorganisms

The AMPLICOR HCV Test, v2.0 was evaluated by testing for potential cross-reactivity with, or interference from, certain pathogenic microorganisms and normal epidermal microflora that could be present in specimens. Twenty-four specimens that contained virus (adenoviruses, enteroviruses, herpesviruses, or retroviruses) and four that contained bacteria (*Propionibacterium acnes*, *Staphylococcus aureus*, or*S. epidermidis*) yielded Negative HCV and Positive HCV IC results with the AMPLICOR HCV Test, v2.0.

The assay was also evaluated for cross-reactivity by testing specimens that represented certain manifestations of infection with *Hepatitis A virus* (HAV) or *Hepatitis B virus* (HBV). Analytes known to be present in these specimens were HAV, IgM antibodies to HAV, HBsAg, or HBsAg with HBeAg. All tested serum and plasma specimens yielded Negative AMPLICOR HCV Test, v2.0 results.

9.5. Endogenous Substances

Serum specimens containing elevated concentrations of albumin, bilirubin, hemoglobin, triglycerides, and immunoglobulins were tested for interference with the AMPLICOR HCV Test, v2.0. These specimens were tested neat or after spiking with a near-LOD concentration of HCV RNA (100 *IU*/mL). Each specimen was tested in triplicate.

Specimens not spiked with HCV yielded Negative results, except for 2 of 11 specimens that contained elevated, or nearly elevated levels, of bilirubin. Negative AMPLICOR HCV Test, v2.0 results from the other 9 elevated-bilirubin specimens indicated lack of interference leading to False Positive results.

All HCV-spiked specimens yielded Positive results, indicating a lack of interference..

9.6. Therapeutic Drugs

Drugs for infectious diseases, or for several conditions associated with hepatitis C therapy, were evaluated for their potential to interfere with the AMPLICOR HCV Test, v2.0. Among the former were drugs for hepatitis C, hepatitis B, HIV, influenza A, or cytomegalovirus-associated syndromes. Each drug was spiked into two plasma concentrations, peak and 3-times peak (1X and 3X C_{max}), that contained HCV at near-LOD (100 *IU*/ml) or was HCV RNA-negative. Specimens were tested in triplicate, with and without spiked drugs. Evaluated drugs did not yield False Positive or False Negative results. At each drug concentration, all HCV-containing (100 *IU*/ml) specimens yielded Positive results.

10. Summary of Clinical Studies

These studies are summarized in the Expected Values and Clinical Evaluation sections of the AMPLICOR HCV Test, v2.0 Package Insert. Certain findings are also summarized below.

10.1. Specimens from Characterized Patients

A prospective study was conducted at four US hepatology centers to evaluate clinical performance of the AMPLICOR HCV Test, v2.0 against results of testing for anti-HCV, and against a combination of anti-HCV results, serum ALT levels, and histologic findings in liver tissue. Studied patients were being investigated for evidence of liver disease, infection with HCV, or both. Patients were excluded if they had undergone liver transplantation or received antiviral therapy for hepatitis C within 6 months of study screening. While HCV RNA was not characterized via quantification or genotyping, the patients were assumed to represent US populations with active HCV infection and chronic liver disease; i.e., range of HCV RNA concentrations (majority likely > 10^4 *IU*/mL) and HCV genotypes (majority of genotype 1 viruses, with genotypes 2 and 3 comprising most of the remainder).

10.2. Expected Values

Specimens from patients with antibody evidence of HCV infection, not treated for hepatitis C: During the clinical study, the vast majority of such specimens yielded AMPLICOR HCV Test, v2.0 absorbance values ($A_{450} \geq 2.0$) much greater than those of the

Equivocal Zone ($0.3 \leq A_{450} < 1.0$): 92% of frozen or refrigerated serum and 93% of frozen EDTA plasma. Few specimens required repeat testing because of a Potentially Inhibited result (0.7% of frozen or 0% of refrigerated serum and frozen EDTA plasma), or an Equivocal Zone result (0.5% of frozen or 0% refrigerated serum and frozen EDTA plasma).

Run failures: Among 101 runs of the AMPLICOR HCV Test, v2.0 during the clinical study, 6 (6%) failed because Positive Controls were out of range ($A_{450} < 1.5$) and 1 (1%) failed because Negative Controls were out of range ($A_{450} = 0.25$). In addition, there were 2 failed runs due to protocol deviations (i.e., failure to follow procedures for specimen).

10.3. Comparison with Results of Anti-HCV Testing

Studied specimens were from 846 patients, including 153 who had received antiviral therapy for chronic hepatitis C that stopped > 6 months before collecting specimens. While these previously treated patients were included because those with active infections were considered to be virologically representative for determining assay performance, their data do not imply AMPLICOR HCV Test, v2.0 performance for monitoring HCV-associated disease or response to treatment. The 693 untreated patients included >200 who had negative anti-HCV EIA results and/or ALT concentration within reference range and no histopathologic evidence of hepatitis; they were studied for approximating test specificity, but their data do not imply performance for testing of individuals without liver disease or antibody evidence of HCV infections (see Warnings). In particular, Positive AMPLICOR HCV Test, v2.0 results were obtained for three patients who had liver disease other than hepatitis C and for whom there was no evidence, or insufficient evidence, to conclude that they were actively infected with HCV.

Analysis of data from specimens of serum and EDTA plasma demonstrated high concordance with results from anti-HCV testing:

- For specimens with EIA-repeat reactive (RR) and SIA-positive results of anti-HCV testing, the vast majority of AMPLICOR HCV Test, v2.0 results were Positive for both matrices: 94% for serum and 92% for EDTA plasma.
- For EIA-RR / SIA-indeterminate specimens, AMPLICOR HCV Test, v2.0 results were Positive for 66% of serum specimens and 50% of EDTA plasma specimens.
- For EIA-negative samples, the vast majority of AMPLICOR HCV Test, v2.0 results were Negative for both matrices (97% for serum and 96% for plasma).

10.4. Comparison with Anti-HCV, ALT, and Histologic findings

Test performance was further evaluated in the subset of patients from whom liver tissue had been collected in the past. AMPLICOR HCV Test, v2.0 results were compared with the combination of anti-HCV results, ALT concentrations, and histologic findings.

Patients with elevated ALT levels and histopathologic evidence of hepatitis: AMPLICOR HCV Test, v2.0 results were Positive for 99% of serum and 97% of EDTA plasma specimens that were anti-HCV EIA-RR and SIA-positive. AMPLICOR HCV Test, v2.0 results were also Positive for all 8 EIA-RR / SIA-indeterminate specimens. Negative

results were obtained with all 17 specimens from EIA-negative patients; their histopathologic characteristics were suggestive of autoimmune hepatitis, hepatitis B, or other forms of non-C hepatitis.

Patients with normal ALT levels and histopathologic evidence of hepatitis: AMPLICOR HCV Test, v2.0 results were Positive for 95% of serum and 87% of EDTA plasma specimens among EIA-RR specimens (one of which was SIA-indeterminate; the others were SIA-positive). AMPLICOR HCV Test, v2.0 results were Negative for all 9 EIA-negative sera from patients with histologic features of hepatitis that were suggestive of diseases other than hepatitis C.

Patients with elevated ALT levels and no histopathologic evidence of hepatitis: AMPLICOR HCV Test, v2.0 results were Positive for 5 of 7 serum specimens, and both EDTA plasma specimens, with EIA-RR / SIA-positive anti-HCV results. AMPLICOR HCV Test, v2.0 results were Negative for 97% of EIA-negative serum specimens.

Patients with normal ALT levels and no histopathologic evidence of hepatitis: Positive AMPLICOR HCV Test, v2.0 results were obtained from (i) 2 of 4 serum specimen and the single EDTA plasma specimen with EIA-RR / SIA-positive results, and (ii) the single EIA-RR / SIA-indeterminate sample. AMPLICOR HCV Test, v2.0 results were Negative for 97% of serum specimens and for the one EDTA plasma specimen from EIA-negative patients. Histopathologic liver disease was evident in these cases (but without features of hepatitis).

10.5. Reproducibility

The AMPLICOR™ HCV Test, v2.0 Package Insert summarizes a reproducibility study that was performed in three clinical laboratories.

11. Conclusions Drawn from the Studies

11.1. Non-Clinical Studies

Analytical Sensitivity

Regardless of the approach to analyzing submitted data, LODs with the WHO International Standard for HCV genotype 1 RNA were ≤ 2.0 $\log_{10}$ IU/mL, an HCV RNA concentration that is much lower than those expected for most patients for whom the AMPLICOR HCV Test, v2.0 is indicated. (Similar data were obtained with other genotype 1 viruses in a reproducibility study that was performed in clinical laboratories: see Package Insert.)

Current technical limitations made it difficult to determine LODs for non-1 genotypes: in particular, there are no international reference materials for recognized HCV genotypes 2-6 and practical quantifying methods have approximately ± 0.3 $\log_{10}$ imprecision. While these limitations prevented LOD determinations from studies with RNAs representing non-1 HCV genotypes, the data were not inconsistent with those from the WHO Standard (i.e., the data were suggestive of LODs within 1 $\log_{10}$ of those for genotype 1). These data also demonstrated that the AMPLICOR HCV Test, v2.0 was likely to detect HCV genotypes 2-

6 at much lower RNA concentrations that those expected for most patients for whom the assay is indicated.

These findings are evidence of the safety and effectiveness of the AMPLICOR HCV Test, v2.0 for its indicated use. In addition, data from an internationally recognized reference material, the WHO International Standard for HCV genotype 1 RNA, is appropriate and essential for enabling comparison among HCV RNA assays.

Analytical Specificity

Potential Cross-reactivity or Interference

Submitted data yielded no evidence of AMPLICOR HCV Test, v2.0 cross-reactivity with, or interference from, most of the analytes that were tested, including certain microorganisms, serum proteins, and pertinent therapeutic drugs.

The tested endogenous substances were shown not to interfere with the AMPLICOR HCV Test, v2.0, with the exception of certain specimens containing bilirubin, which might have yielded False Positive results. Among 11 specimens tested for analytical specificity in the presence of elevated concentrations of bilirubin and without spiked HCV, 2 did not yield Negative AMPLICOR HCV Test, v2.0 results. It could not be determined if the Positive and Equivocal results from these 2 specimens represented accurate detection of HCV RNA because anti-HCV results were not available. These concerns are noted in the procedural limitations section. Non-clinical studies of the AMPLICOR HCV Test, v2.0 did not include evaluation of potential interference with detection of low HCV RNA concentrations by other analytes that often circulate at elevated concentrations during viral hepatitis. While there was no apparent effect of ALT on frequencies of Positive results during the clinical study, it was assumed that most studied patients had $>10^4$ *IU*/mL of HCV RNA; furthermore, most specimens were frozen (which reduces concentrations of these analytes) before AMPLICOR HCV Test, v2.0 testing. These concerns are noted in the procedural limitations section.While tested therapeutic drugs did not appear to interfere with the ability to detect HCV RNA, specimens were spiked with each drug and so the study did not provide data about drug metabolites that might affect assay performance. In addition, non-clinical studies did not evaluate potential effects of anti-bacterial or anti-fungal drugs that are frequently administered to patients. FDA did not recommend any pertinent post-approval studies.

Cross Contamination

Cross contamination is a recognized problem for all laboratory assays, and particularly those based on target amplification occurred. Submitted data indicated infrequent (≤1.2%) cross-contamination rates during studies of the AMPLICOR HCV Test, v2.0.

11.2. Clinical Studies

Study of patients with liver disease

The study of patients with liver disease demonstrated high concordance between AMPLICOR HCV Test, v2.0 results and those from anti-HCV testing. There was also high

concordance between AMPLICOR HCV Test, v2.0 results and the combination of serologic, biochemical and histologic findings. Such concordance was plausible because study data indicated that most of the studied patients were likely to have had chronic liver disease. Peer-reviewed scientific literature had previously demonstrated that (i) most HCV infections persist with detectable HCV viremia, particularly in individuals with elevated serum concentrations of ALT; and (ii) EIA-negative HCV infections with detectable RNA are very unusual, especially in a chronically infected population.

Limits of current technology and design of the study made it difficult to determine the true frequencies of false AMPLICOR HCV Test, v2.0 results. That is, while there may have been unrecognized False Positive and False Negative AMPLICOR HCV Test, v2.0 results, FDA decided that the study data were appropriate for contributing to the determination of safety and effectiveness. Most importantly, there is no reference assay with known accuracy for detecting HCV RNA.

Study design did not include determination of HCV genotypes or HCV RNA concentrations. While it could not be determined if these variables affected AMPLICOR HCV Test, v2.0 performance, FDA recognized the absence of reference assays for genotyping or quantifying HCV RNA and accepted the assumption that these variables were adequately represented among enrolled patients at the four US study sites. Furthermore, non-clinical studies demonstrated that any effects of genotype were unlikely at the high concentrations of HCV RNA ($>10^4$ *IU*/mL) that most patients in this study were assumed to have had.

The data from this clinical study demonstrated that AMPLICOR HCV Test, v2.0 yielded Positive results for a very high proportion of patients with liver disease and antibody evidence of HCV infection. The assay also yielded Negative results for a very high proportion of patients who had liver disease without anti-HCV (these data do not imply performance for testing of individuals without liver disease or antibody evidence of HCV infections). Although the exact proportions of false results could not determined, FDA decided that the data provided reasonable assurance of AMPLICOR HCV Test, v2.0 safety and effectiveness for diagnosis of active HCV infection among patients with evidence of liver disease and antibody evidence of HCV infection. Effectiveness of the AMPLICOR HCV Test, v2.0 was also supported by the low frequencies, during the clinical study, of failed runs and of Potentially Inhibited and Equivocal results that require repeat testing.

Reproducibility

Performance of the AMPLICOR™ HCV Test, v2.0 assay was reproducible among studied variables that included reagent lots, study sites, and days, and sample matrices (See package insert).

11.3. Risk / Benefit Analysis

The presence of HCV viremia, as determined by detecting HCV RNA, is direct evidence of replication of the virus; i.e., active HCV infection. Thus, the most significant benefit is that AMPLICOR HCV Test, v2.0 results can provide evidence of active HCV infection for patients who have evidence of liver disease and antibody evidence of HCV infection.

(Antibody evidence of HCV infection is defined here as repeatedly reactive results from an anti-HCV EIA alone, or in combination with positive or indeterminate results from an anti-HCV SIA).

Available data indicate that the likelihood of benefit, to patients tested by the AMPLICOR HCV Test, v2.0, exceeds any known or potential adverse event or risk to patients or laboratory personnel.

The clinical and non-clinical laboratory studies described above provided reasonable assurance that the AMPLICOR HCV Test, v2.0 can be safely and effectively used for qualitative detection of HCV RNA in human serum or EDTA plasma. These studies also provided scientific evidence of diagnostic benefits to be gained from FDA approval for marketing of the AMPLICOR HCV Test, v2.0.

11.4. Safety

As a diagnostic test, the AMPLICOR™ HCV Test, v2.0 assay involves removal of blood from an individual for testing purposes. The test, therefore, presents no more safety hazards to and individual being tested than other tests where blood is removed.

12. Panel Recommendations

The Microbiology Devices Advisory Panel (MDAP) met on July 28, 2000 to consider safety and effectiveness of the AMPLICOR HCV Test, v2.0. MDAP recommended approval subject to the following conditions:

1) RMS should submit, and FDA should satisfactorily analyze, appropriate data for determining the assay's reproducibility.

2) The Package Insert should include revisions to the intended use, warnings, precautions and performance sections.

13. CDRH Decision

CDRH concurred with MDAP recommendations. RMS provided additional data, analyses, and peer-reviewed scientific literature to address assay reproducibility and other issues. Certain issues were rectified by appropriate data that RMS submitted in the Amendments, by FDA analyses of RMS data and peer-reviewed scientific literature, or by warnings and limitations in the Package Insert (Labeling restrictions). Other issues were rectified by deciding that the probability of unrecognized inaccurate performance or adverse effects was exceeded by public health importance of the first approval for US marketing of an HCV RNA assay. The Package Insert was revised by using the MDAP recommendations.

On October 6, 2000, the applicant's two manufacturing facilities were found to be in compliance with the Quality Systems Regulation (21 CFR 820).

Having determined that there was reasonable assurance of safety and effectiveness for the AMPLICOR HCV Test, v2.0, CDRH issued an approval order on July 3, 2001.

14. Approval Specifications

Directions for Use: see Package Insert and other Labeling

Conditions of Approval: CDRH Approval of this PMA is subject to full compliance with the conditions described in the approval order.

Postapproval Requirements and Precautions: See Approval Order

15. References

Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 1999; 341:556-562.

Centers for Disease Control and Prevention. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *MMWR Morb Mortal Wkly Rep* 1998;47 (No. RR-19):1-54. Available at: http://www.cdc.gov/mmwr/PDF/rr/rr4719.pdf.

Gretch, DR. Diagnostic tests for hepatitis C. *Hepatology* 1997; 26 (Suppl 1): 43S-47S.

Schiff ER, de Medina M, Kahn RS. New perspectives in the diagnosis of hepatitis C. *Semin Liver Dis* 1999; 19 (Suppl1): 3-15.

Lok AS, Gunaratnam NT. Diagnosis of hepatitis C. *Hepatology* 1997; 26 (Suppl 1): 48S-56S.

National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. *Hepatology* 1997; 26 (Suppl 1):2S-10S. Available at: http://odp.od.nih.gov/consensus/cons/105/105_intro.htm.

Myers TW, Gelfand DH. Reverse transcription and DNA amplification by a *Thermus thermophilus* DNA polymerase. *Biochemistry* 1991; 30:7661-7666.

Stuyver L, Rossau R, Wyseur A, et al. Typing hepatitis C virus isolates and characterization of new subtypes using a line probe assay. *J Gen Virol* 1993; 74:1093-1102.

Machida A, Ohnuma H, Tsuda F, et al. Two distinct subtypes of hepatitis C virus defined by antibodies directed to the putative core protein. *Hepatology* 1992;16:886-891.

Bukh J, Purcell RH, Miller RH. Importance of primer selection for the detection of hepatitis C virus RNA with the polymerase chain reaction assay. *Proc Natl Acad Sci USA* 1992; 89: 187-191.

Bukh J, Purcell RH, Miller RH. Sequence analysis of the 5' noncoding region of hepatitis C virus. *Proc Natl Acad Sci USA* 1992; 89:4942-4946.

Okamoto H, Okada S, Sugiyama Y, et al. The 5'-terminal sequence of the hepatitis C virus genome. *Jpn J Exp Med* 1990; 60:167-177.

Young KKY, Resnick R, Myers TW. Detection of hepatitis C virus RNA by a combined reverse transcriptase-polymerase chain reaction assay. *J Clin Microbiol* 1993; 31:882-886.

Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* 1990; 93:125-128.

Aoyagi K, Ohue C, Iida K, Kimura T, Tanaka E, Kiyosawa K, Yagi S. Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. *J Clin Microbiol* 1999; 37:1802-1808.

Saldanha J, Lelie N, Heath A. Establishment of the First International Standard for Nucleic Acid Amplification Technology (NAT) Assays for HCV RNA. WHO Collaborative Study Group. *Vox Sang* 1999; 76:149-158.